



United States Army Medical Research
Institute of Infectious Diseases

Preparation of *Burkholderia pseudomallei* Polysaccharide-CRM₁₉₇ Conjugate, a Potential Vaccine Candidate for Glanders and Melioidosis

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Report Documentation Page			Form Approved OMB No. 0704-0188		
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1. REPORT DATE 01 OCT 2005		2. REPORT TYPE N/A		3. DATES COVERED -	
4. TITLE AND SUBTITLE Preparation of Burkholderia pseudomallei Polysaccharide-CRM197 Conjugate, a Potential Vaccine Candidate for Glanders and Melioidosis			5a. CONTRACT NUMBER		
			5b. GRANT NUMBER		
			5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S)			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Bacteriology Division, USAMRIID, Fort Detrick, Frederick, Maryland			8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)			10. SPONSOR/MONITOR'S ACRONYM(S)		
			11. SPONSOR/MONITOR'S REPORT NUMBER(S)		
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release, distribution unlimited					
13. SUPPLEMENTARY NOTES See also ADM001851, Proceedings of the 2003 Joint Service Scientific Conference on Chemical & Biological Defense Research, 17-20 November 2003. , The original document contains color images.					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 15	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

Melioidosis

- Melioidosis is an infectious disease caused by the bacterium *Burkholderia pseudomallei*
- It is most frequently reported in Southeast Asia and Northern Australia
- Melioidosis most commonly involves the lungs where the infection can form a cavity of pus (abscess)
- It can spread from the skin through the blood to affect the heart, brain, liver, kidneys, joints, and eyes
- Patients can have associated headaches, fever, chills, cough, chest pain, and/or loss of appetite

Glanders

- Glanders is an infectious disease that is caused by the bacterium *Burkholderia mallei*
- The types of infection include localized, pus-forming cutaneous infections, pulmonary infections, bloodstream infections, and chronic infections of the skin

Current Status Treatment of Meliodosis and Glanders

- *Burkholderia mallei* and *B. pseudomallei* are the causative agents for glanders and melioidosis, respectively
- Both of these organisms have been considered as potential agents for biological warfare and biological terrorism
- Currently, these infections are treated with antibiotics
- Currently, no vaccines are available for protection against glanders and melioidosis

Strategy for Preparation of Vaccine for Glanders and Melioidosis

- The strategy is to prepare capsular polysaccharides in milligram quantities and employ them as protective antigens
- The next step is to conjugate the polysaccharides to a carrier protein
- Examine the efficiency of conjugation
- Assay the polysaccharide- protein conjugate
- Test the polysaccharide-protein conjugate in a mouse model against aerosol challenge with *B. mallei* and *B. pseudomallei*

Methods

- **Phenol-sulfuric acid method:**
 - To assay total neutral sugars - used to measure polysaccharide-protein conjugate, monitor purification process of polysaccharide from the bacteria
- **Reducing sugars - MBTH/ferric ammonium sulfate method:**
 - To prepare polysaccharide-protein conjugate, the polysaccharide will be coupled to the protein via the reducing group of the polysaccharide
 - The reducing sugar estimation method is employed to test the efficiency of the conjugation procedure

Methods (continued)

- **Acetolysis- 2% HAc, 100° C, 2h:**
 - Cleaves the linkage at KDO in LPS to release lipid A, core oligosaccharides, and O-antigens (repeating sugar units)
- **Western blot:**
 - Polysaccharide-protein conjugate, Antibody produced against the whole cells, capsule, LPS

Rationale for Choosing a Protein Carrier

- To increase the immune response, polysaccharides are usually conjugated to protein carriers
- We chose CRM₁₉₇ as the carrier
- CRM₁₉₇ is diphtheria toxin mutant, commercially available
- CRM₁₉₇ had been conventionally used as a protein carrier for polysaccharide vaccines for example meningococcal polysaccharide vaccine, pneumococcal and Haemophilus influenzae b polysaccharide vaccines (Aventis Pasteur, Merck and Weyth)

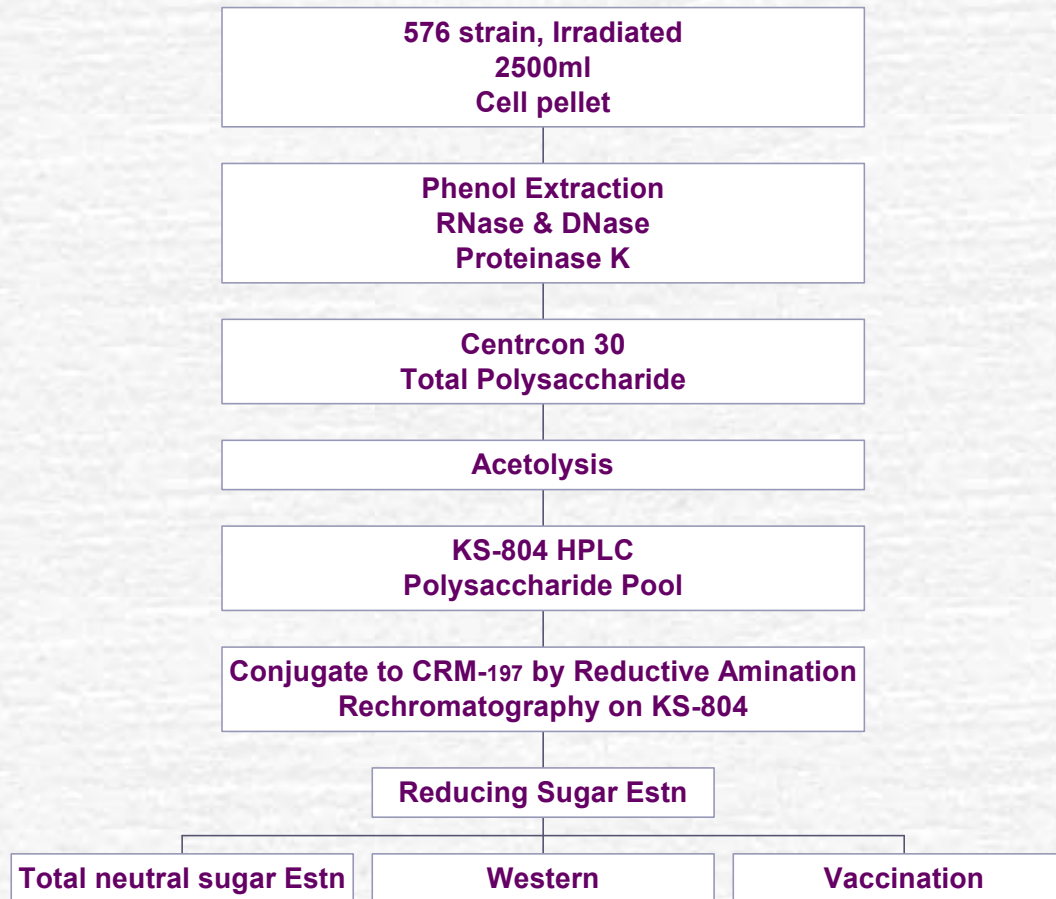
Polysaccharides

- *Burkholderia mallei* and *B. pseudomallei* produce two types of polysaccharides:
 - Capsular polysaccharide - 2-O-acetyl- 6-deoxy manno heptose homopolymer - <200kDa
 - LPS- Heteropolymer of repeating D-glucose and L-talose

Rationale for choosing capsular polysaccharides as vaccine candidates

- Capsule is the virulence factor:
 - Dave DeShazer prepared a capsule mutant (DD3008) and demonstrated that the mouse aerosol LD₅₀ was at least 10³ times greater than the wild type (China7)
- *B. mallei* and *B. pseudomallei*, the pathogenic bacteria produce capsule, while *B. thailandensis* (non pathogenic) does not produce capsule

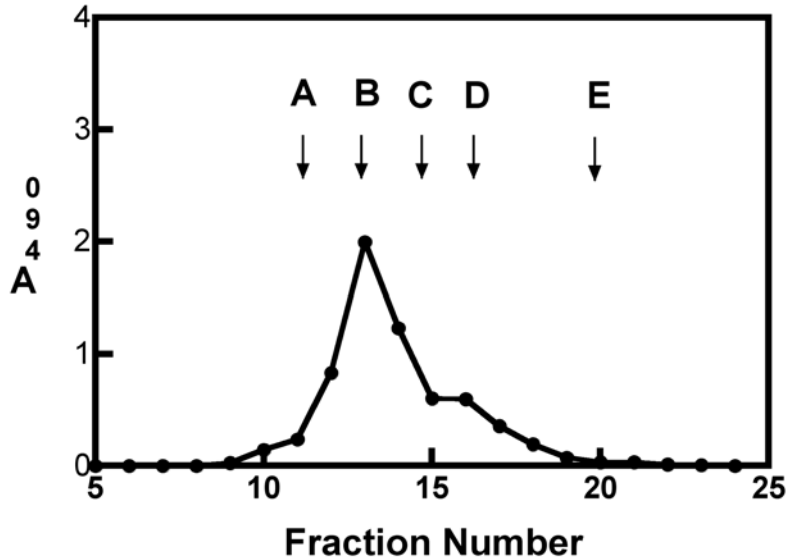
Fractionation Scheme for Polysaccharides



KS-804 HPLC of 576

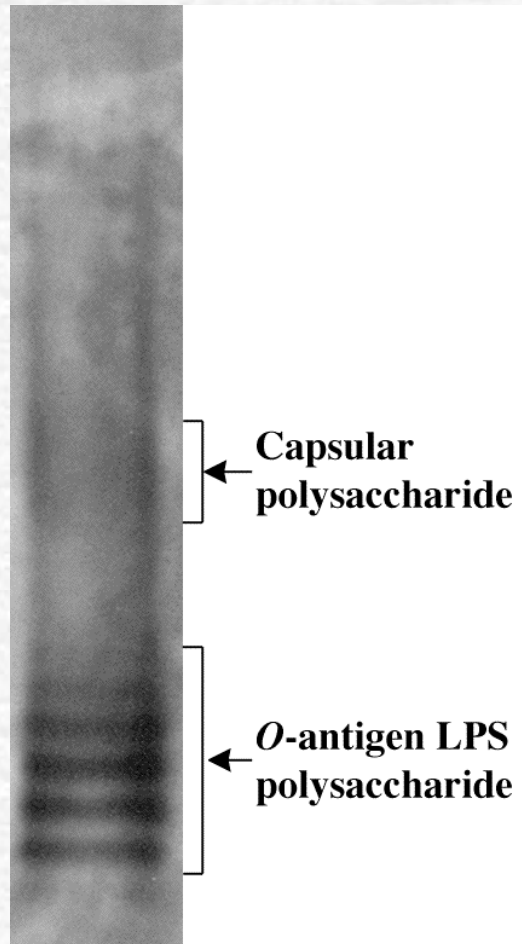
Polysaccharides/Acetolysis/Sugar Phenol-H₂SO₄

100 μ l of Fraction



- A, B, C, D: Pullulan Stds 400, 50, 10, 5 kD
- E: Mannose, MW 180
- Fractions Pooled: #12-14
- Dry weight: 8 mg
- Total neutral sugar content
40 μ mole Glc equivalents, 7.2 mg

Western Blot of 576 Polysaccharide-CRM₁₉₇ Conjugate



- Antigen 576PS - CRM₁₉₇ polysaccharide conjugate
- Ab - 576 whole cells - Polyclonal
- Detection ECL HRP
- MW of the conjugate - > 60kD - 200kD

CONCLUSIONS

- We isolated polysaccharides (capsular and LPS) from *B. pseudomallei* in milligram quantities
- We successfully conjugated the above polysaccharides to a carrier protein CRM₁₉₇
- Western blot analysis of the conjugate indicated that the polysaccharides are derived from the capsule and LPS
- Experiments are in progress to test this polysaccharide-CRM₁₉₇ conjugate for protection against glanders and melioidosis.

Acknowledgements

- Dave DeShazer
- Dave Waag
- Marilyn England
- SPC Rodjimir Barraiz

- **Disclaimer**
 - Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.
 - The research was sponsored by the Medical Biological Defense Research Program, U.S. Army Medical Research and Materiel Command – (Project # 03-4-5X-029).